REMARKS

Interview Regarding the Invention

The undersigned and applicants greatly appreciate the examiner taking the time to discuss this application. As discussed with the examiner, the invention is the development of a bacterial strain that overcomes a long standing problem in the field:

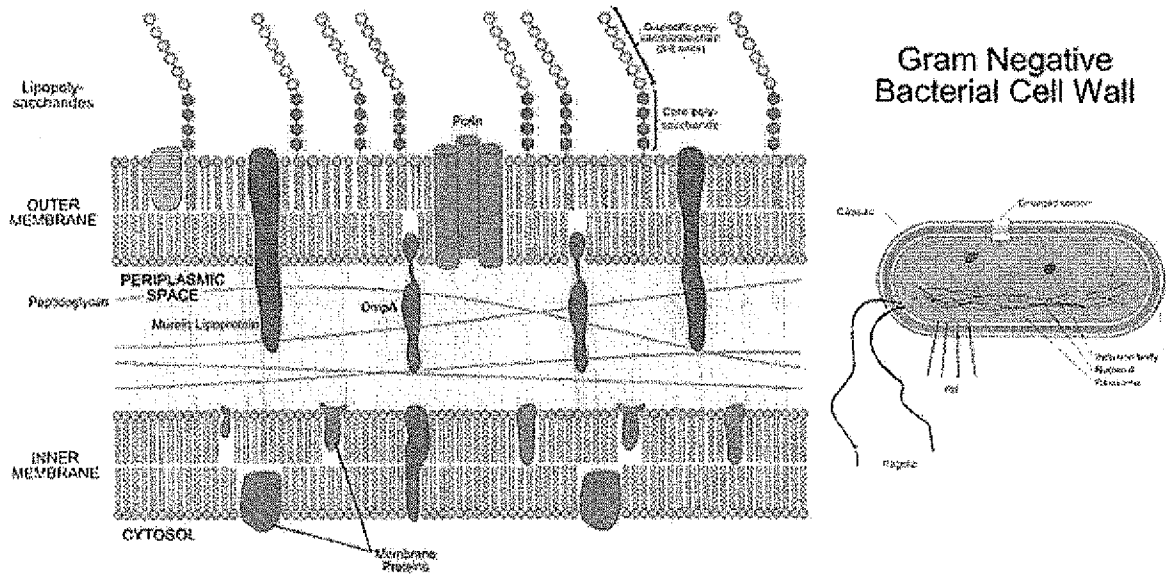
How to economically separate the polyhydroxyalkanoate from the lysed bacteria after fermentation.

The prior art separated the polyhydroxyalkanoate, which is present as part of a thick, highly viscous mixture of cell debris, nucleic acid, protein, and other materials after lysis, by addition of an exogenous nuclease. This was effective, but expensive.

The solution is elegant:

Make the polyhydroxyalkanoate in a bacteria with a periplasmic space, rather than in a gram positive bacteria, so that the expressed nuclease is sequestered in large amount in the periplasmic space.

A periplasmic space is the space *within* the wall of a gram negative bacteria, as shown in the following diagram from Wikipedia.



A gram positive bacteria does not have a double layered wall like a gram negative bacteria so there is no periplasmic space. *Ralstonia eutropha, Methylobacterium organophilum, Methylobacterium extorquens, Aeromonas caviae, Azotobacter vinelandii, Alcaligenes latus, Pseudomonas oleovorans, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas acidophila, Pseudomonas resinovorans, Escherichia coli,* and *Klebsiella* are examples of gram negative bacteria.

When a gram negative bacteria makes large quantities of nuclease, it is retained within the periplasmic space since the bacteria does not have the ability to secrete most or all of it.

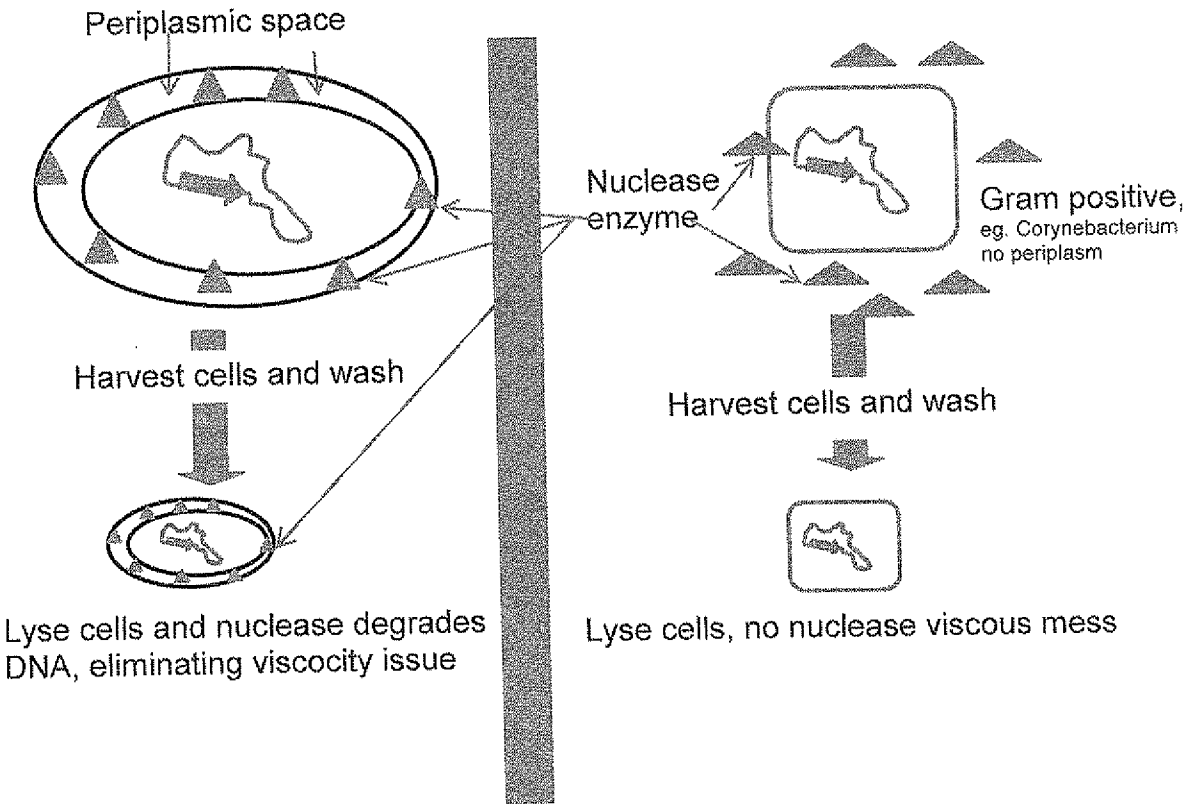
This has two important advantages:

(1) the accumulating nuclease does not kill the host cell (not an issue with gram positive that naturally secrete the nuclease into the cell culture medium rather than accumulate the nuclease intracellularly); and

(2) the accumulated nuclease is not washed away when the cells are washed before lysing (unlike the case with gram positive cells that secrete nuclease into the cell culture medium), so it is released when the cells are lysed and is available to degrade the cellular material.

The diagram below depicts nuclease production in gram positive and gram negative bacteria.

GRAM NEGATIVE BACTERIA GRAM POSITIVE BACTERIA



None of the prior art recognizes that the use of the gram negative organism in combination with expression of high levels of nuclease, which then accumulates within the periplasmic space, provides a significant economic advantage.

Rejections Under 35 U.S.C. § 103

Claims 1, 3, 4, 6 and 7 were rejected under 35 U.S.C. § 103(a) as obvious over WO 94/10289 by Greer, *et al.*, ("Greer"), Atkinson, *et al.*, Biochemical Engineering and Biotechnology Handbook, 2nd Edition, Stockton Press: New York, 1991 ("Atkinson") and Lee, *et al.*, *Adv. Biochem. Eng. Biotechnol.* 52:27-58 (1995) ("Lee"), or Miller, *et al.*, *J. Bacteriology* 169(8):3508-3514 (1987) ("Miller") in view of Liebl et al., *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl"), or Miller. Applicants respectfully traverse this rejection.

The Legal Standard

When applying 35 U.S.C. § 103, the following tenets of patent law must be adhered to:

(a) determining the scope and contents of the prior art;

(b) ascertaining the differences between the prior art and the claims in issue;

(c) resolving the level of ordinary skill in the pertinent art; and

(d) evaluating evidence of secondary consideration.

Graham v. John Deere, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459, 467 (1966). These four factors are traditionally referred to as the Graham factors.

Obviousness is a legal conclusion. *See Graham v. John Deere Co.*, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459 (1966). The *Graham* analysis was recently affirmed by the Supreme Court in *KSR Int'l Co. v. Teleflex, Inc.*, 127 S. Ct. 1727, 82 U.S.P.Q.2d 1385 (2007).

The obviousness analysis requires looking at the invention as a whole. "Focusing on the obviousness of substitutions and differences, instead of on the invention as a whole, is a legally

improper way to simplify the often difficult determination of obviousness." *Gillette Co. v. S.C. Johnson & Sons, Inc.*, 919 F.2d 720, 724, 16 U.S.P.Q.2d 1923 (Fed. Cir. 1990); *see Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1383, 231 U.S.P.Q. 81, 93 (Fed. Cir. 1986).

Hindsight analysis, such as picking and choosing from prior art references using the claimed invention as a template, has long been forbidden. *See, e.g., In re Fine*, 837 F.2d 1071, 1075 (Fed. Cir. 1988), which states that "One cannot use hindsight reconstruction to pick and choose among isolated disclosures on the prior art to deprecate the claimed invention." In *KSR*, the Court also warned against the use of hindsight analysis in making an obviousness determination. The Court stated, "A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning." (*KSR*, 127 S. Ct. at 1742, citing *Graham*, 383 U.S. at 36 (warning against a "temptation to read into the prior art the teachings of the invention in issue" and instructing courts to "guard against slipping into the use of hindsight" (quoting M*onroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406, 412, 141 U.S.P.Q. 549 (6th Cir. 1964))).

Analysis

None of the References cited by the Examiner alone or in combination, disclose the claimed bacterial straing

The Examiner's comments are either directed to limitations not recited in the claims, or partial consideration of the claim limitations

Claim 1 as amended defines a bacterial strain producing polyhydroxyalkanoates, wherein the bacteria express a heterologous nuclease gene or a genetically modified homologous nuclease

gene, the product of which is accumulated in the periplasmic space in an amount effective to degrade at least 95% of all of the nucleic acid released following lysis of cells in the growth medium in less than 24 hours so that recovery of the product is enhanced.

The Examiner has not identified disclosure in any of the references cited which shows expression of nuclease in a gram negative bacteria having a periplasmic space so that the nuclease accumulates in the periplasmic space. Thus, the Examiner has not established a *prima facie* case of obviousness.

The Examiner asserts that one would have been motivated to engineer a bacterial strain to express *Staphylococcal aureus* nuclease as taught by Liebl or Miller, or a homologous nuclease gene that has been modified to enhance nuclease activity, so that this bacterial strain would produce and excrete the nuclease into the *bacterial growth medium* as part of a fermentation process. However, the claims require that the nuclease is secreted *into the periplasmic space*, not the growth medium. The references alone or in combination do not disclose secretion of nuclease into the periplasmic space of gram negative bacteria and therefore do not meet all of the claimed limitations.

The Examiner's assertion that Alcaligenes eutrophus expresses a homologous nuclease gene is unsupported by any evidence

The Examiner's attention is drawn to the present specification at least at Example 3, which shows that of the 10 transgenic *R. eutropha* (*Alcaligenes eutrophus)* colonies selected after conjugation with a host *E. coli* strain harboring the nuclease plasmid for integration, only one culture produced an active nuclease that was localized in the periplasm of the transgenic strain. This evidence clearly rebuts the statements made by the examiner: the art does not disclose such an organism as defined by the claim, the likelihood of it existing in nature without (1) the motivation to make it and (2) screening to identify the few out of many that would not have the claimed properties. There is simply no evidentiary support for the assertion that *Alcaligenes eutrophus* expresses homologous nuclease which is secreted into the periplasmic space.

The combined references do not provide any expectation of success in arriving at the claimed process

The Examiner asserts that one would have a reasonable expectation of success in arriving at the bacteria required by the claimed process because both Liebl and Miller were able to express nuclease in different bacterial species. However, the Examiner did not provide any reasons why merely expressing nuclease in bacteria as disclosed in Lieble and Miller provides an expectation of success in arriving at bacteria exporting nuclease to the periplasmic space, since neither of Liebl or Miller is concerned with engineering bacteria to export nuclease into the periplasmic space . Release of nuclease into the culture medium is not release into the periplasmic space as required by the claims. Further, when the nuclease is released into the cell

culture medium, the bacteria inherently cannot meet the limitation that the nuclease is exported into the periplasmic space in an amount effective to degrade at least 95% of all of the nucleic acid released following lysis of cells. There is no nuclease in the periplamic space, when the nuclease is secreted into the culture medium. Therefore, neither of Liebl or Miller can provide an expectation of success that the claimed method would work.

See, in particular, the data on page 15, Table 1 - out of **50 nuclease positive cells, only a very few showed high level of nuclease activity. This is a critical feature of the process using the claimed bacterial strain, required for scale up for commercial production of polymer.**

As discussed above, the claimed process presents two advantages which are not contemplated or disclosed in the cited art; (i) exporting the nuclease into the periplasmic space sequesters the expressed nuclease until it is released when desired during the fermentation process, by cell lysis, and (ii) sequestering the nuclease in the periplasmic space protects the cells themselves from the toxic effects of nuclease, had it been secreted into the culture medium, ***allowing accumulation of significantly higher levels than can be achieved in gram positive cells***. The combination of references cited by the Examiner cannot provide a motivation to modify the disclosures therein in order to arrive at the claims or an expectation of success in arriving at the claimed process, where the prior references combined do not contemplate or disclose all of the claimed elements. For at least these reasons, the claims as amended are non-obvious over the combination of the prior art.

In summary, the prior art cited by the examiner fails to teach genetic engineering of an organism to export a nuclease into the periplasmic space. The evidence in the specification establishes that even when intentionally engineered, an intense selection process is required to identify organisms expressing sufficient nuclease to degrade the majority of nucleic acid released when the cells are lysed. This is not taught by the prior art.

Allowance and rejoinder of claims 1, 3, 4, 6, 7, 11, 12, 14-16, 19 and 21 is respectfully solicited. Claims 11, 12, 14-16 and 19 are related to claims 1, 3, 4, 6-9 and 14-16 as product and process of use. Accordingly, no new search would be required should claims 1, 3, 4, 6 and 7 be found to be allowable.

Respectfully submitted,

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Date: September 22, 2010

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